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(71) Applicants: ALPHA 1 BIOMEDICALS, INC. [US/US]; Two Democracy Center, Suite 1200, 6903 Rockledge Drive, Bethesda, MD 20817-1818 (US). THE GEORGE WASHINGTON UNIVERSITY MEDICAL CENTER [US/US]; 2300 Eye Street, N.W., Washington, DC 20037 (US).

(72) Inventors: CROCKFORD, David, R.; 113 Record Street, Frederick, MD 21701 (US). RUBIN, Bruce, K.; 1850 North Signal Hills Drive, Kirkwood, MO 63122 (US). BERMAN, Michael, L.; 8824 Watts Mine Terrace, Potomac, MD 20854 (US). GOLDSTEIN, Allan, L.; 6407 Bradley Boulevard, Bethesda, MD 20817 (US). BAUMAN, Christian; 14355 Georgia Avenue, Silver Spring, MD 20906 (US). KATER, Amon; 950-25th Street, N.W., 514 South, Washington, DC 20037 (US).

(74) Agents: REPPER, George, R. et al.; Rothwell, Figg, Ernst & Kurz, 555 13th Street, N.W. #701 East, Washington, DC 20004 (US).

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(54) Title: TREATMENT OF OBSTRUCTIVE AIRWAY DISEASE BY ADMINISTERING THYMOSIN β_4 OR COADMINISTRATION OF THYMOSIN β_4 AND DNase I

(57) Abstract

A method of treating obstructive airway disease (OAD) such as cystic fibrosis involves contacting OAD sputum with a viscoelasticity-reducing amount of Thymosin β_4, or a combination of Thymosin β_4 and DNase I.

TREATMENT OF OBSTRUCTIVE AIRWAY DISEASE BY ADMINISTERING THYMOSIN B4, OR COADMINISTRATION OF THYMOSIN B4 AND DNase I

The present invention relates to methods and compositions for treating obstructive airway disease in mammals.

Description of Background Art

Obstructive airway disease (OAD) encompasses a number of respiratory disorders and is associated with viscoelastic secretions or exudate (sputum) in the patient's airways which contribute significantly to respiratory distress and may also contribute to progressive lung destruction.

OAD sputum is a complex material known to contain DNA and other materials, including proteins such as actin. OAD sputum is produced in patients with cystic fibrosis (CF), and may also be produced in patients with various forms of bronchitis, bronchiolitis, pneumonia, asthma, sinusitis, bronchorrhea, adult respiratory distress syndrome (ARDS), empyema, bronchiectasis, bronchocoele and emphysema.

Recombinant human DNase I (rhDNase I) has been reported to diminish viscosity of CF sputum *in vitro* (Shak et al., *PNAS USA*, *87*:9188-9192 [1990]). Human DNase I has been approved in the United States for treating certain CF patients.

Thymosin β_4 ($T\beta_4$) is a peptide which has been reported as containing 43 amino acids. Amino acid sequence information on $T\beta_4$ is disclosed in U.S. Patent No. 4,297,276, herein incorporated by reference.

$T\beta_4$ has been found to be present in numerous tissue types in mammals and has also been implicated in a wide variety of cellular and physiological processes including actin sequestration within cells, inducing terminal deoxynucleotidyl transferase activity of bone marrow cells, stimulating secretion of hypothalamic luteinizing hormone releasing hormone and luteinizing

Fig. 9 is a bar graph showing the effect of $T\beta_4$ and DNase I on the vectorial sum of storage modulus and loss modulus of internal OAD sputum at 1 radian/sec.

Fig. 10 is a bar graph showing the effect of $T\beta_4$ and DNase I on OAD sputum loss modulus at 100 radian/sec.

Description of the Preferred Embodiments

One embodiment of the present invention involves administration of $T\beta_4$ to mammals to treat OAD including respiratory disorders such as acute and chronic respiratory distress syndromes, chronic bronchitis, asthma, emphysema and cystic fibrosis. Without being bound to any particular theory, it is believed that these respiratory disorders may be associated with excess actin polymerization, i.e., polymerization of G-actin (monomeric form) into F-actin.

The terms "Thymosin β_4" and "$T\beta_4$" refer to peptides having the amino acid sequence disclosed in U.S. Patent No. 4,297,276, supra.

According to one aspect of the present invention, effective amounts of $T\beta_4$ are administered to a mammal, such as a human patient having a respiratory disorder, so as to depolymerize F-actin, or alternatively prevent G-actin polymerization. Such effective amounts can be referred to as actin-antipolymerizing amounts.

Thus, $T\beta_4$ can be utilized in accordance with the present invention to treat respiratory disorders mediated by excess actin polymerization. Accordingly, $T\beta_4$ can be utilized to treat patients having a respiratory disorder selected from the group consisting of acute and chronic respiratory distress syndromes, and advantageously can be utilized to treat chronic bronchitis, asthma, emphysema and cystic fibrosis.

A preferred embodiment of the present invention involves treating cystic fibrosis with $T\beta_4$. Patients with cystic fibrosis accumulate thick secretions (sputum) in their airways that cause progressive pulmonary destruction. Cystic fibrosis sputum is a complex material, but a major cause of its thick consistency is pus, derived from masses of degenerating leukocytes.

The formulations include those suitable for inhalation, injection/infusion (including parenteral, subcutaneous, intramuscular, intravenous and intradermal) or other routes of administration. The formulations may conveniently be presented in unit dosage form, including aerosol, liquid, solid, or powered unit dosage form, and may be prepared by any suitable pharmaceutical method.

Such methods include, but are not limited to, the step of bringing into association $T\beta_4$ with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association $T\beta_4$ with liquid carriers or finely divided solid carriers or both.

Formulations of the present invention suitable for oral administration may be presented as discrete units each containing a predetermined amount of $T\beta_4$; as an aerosol; as a powder; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion, etc.

Aerosols suitable for inhalation generally contain liquid or solid particles of less than about 100 microns in size, preferably less than about 50 microns in size, and more preferably less than about 25 microns in size. In particularly preferred embodiments, the aerosol particle size is in the range of about 0.1-10 microns, more preferable less than about 4 microns, and most preferable about 0.1-3 microns.

Formulations suitable for injection/infusion, or parenteral administration, include aqueous and non-aqueous sterile injection solutions which may optionally contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with body fluids of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use.

and may contain anti-oxidants, buffers, bacteriostats, antibiotics, solutes, and/or other ingredients.

In particularly preferred embodiments, the inventive pharmaceutical formulation including $T\beta_4$ and DNase I is administered to an OAD patient by introducing the formulation into one or more airways of the patient so as to contact the formulation with OAD sputum present in the patient's airways. Preferred methods of administration including inhalation of the inventive pharmaceutical formulation into the patient's lungs through the patient's mouth and/or nose. In this embodiment, the inventive formulation can be an aerosol. Injectable or infusible compositions may also be administered, either concurrently, separately or alone.

Effective amounts of $T\beta_4$ are amounts sufficient to depolymerize F-actin in OAD sputum or, alternatively, prevent G-actin polymerization in OAD sputum. Such effective amounts can be referred to as actin-antipolymerizing amounts.

Effective amounts of DNase I are capable of further reducing the viscoelasticity of OAD sputum in conjunction with $T\beta_4$ by cleaving elongate strands of DNA present in OAD sputum and/or further preventing polymerization of actin. $T\beta_4$ may also enhance DNase I activity in cleaving DNA by preventing DNase I from binding to actin.

In preferred embodiments, the $T\beta_4$ and DNase I are each present in the inventive pharmaceutical formulation in a respective ratio of from about 1:2 to about 2:1, more preferably at a ratio of about 1:1.

In accordance with one embodiment, the concentration of $T\beta_4$ in the inventive formulation is within a range of about 0.1-200 mcg/ml, preferably about 0.3-150 mcg/ml, more preferably about 0.5-30 mcg/ml, even more preferably about 1-10 mcg/ml, still more preferably about 2-5 mcg/ml, and most preferably about 3 mcg/ml.

The concentration of DNase I in the inventive formulation can be within a range of about 0.1-200 mcg/ml, preferably about 0.3-150 mcg/ml, more

substantially similar to that of native $T\beta_4$. Representative sequences are shown in Fig. 1.

The invention also is applicable to native (*i.e.*, naturally-occurring) human DNase I, as well as other DNase I peptides which are compatible with human patients, along with synthetic DNase I and recombinant DNase I having an amino acid sequence of native DNase I, biologically-active amino acid sequences substantially similar thereto, or a biologically-active abbreviated sequence form thereof, and their biologically-active analogs (including muteins) having substituted, deleted, elongated, replaced or other modified sequences which posses bioactivity substantially similar to that of human DNase I.

The following examples are for illustrative purposes only, and are not to be construed in a limiting sense.

Example 1

Synthetic $T\beta_4$ was provided by Alpha 1 Biomedicals, Inc. (Two Democracy Center, 6903 Rockledge Drive, Ste. 1200, Bethesda, Maryland 20817). $T\beta_4$ was prepared by solid phase peptide synthesis.

Methods

CF Sputum Viscosity Assay

For measuring the difference in viscosity between the samples incubated with $T\beta_4$ and water an apparatus was utilized that was used in a sliding assay which measured a rate of migration of sputum samples that were treated with varying amounts of $T\beta_4$ and corresponding water controls. The apparatus was a grooved plastic surface that could lie in a flat position and upon addition of samples be turned upright at a right angle and the sliding of the sample was measured (a modified tube gel casting stand). The surface was coated with silicon-spray to compensate for any variations of the surface of the apparatus. The migration distance of the apparatus was 6.9 cm.

Each sample contained 100 ug of sputum. The sputum was spread on a plate and the 10 ug samples were cut and weighed and placed in a siliconized

(F-actin) was incubated with varying amounts of $T\beta_4$ protein. $T\beta_4$ was allowed to incubate with F-actin for 1 hour at room temperature. After each of the sample incubations, 10 ul of DNaseI was added to a quartz cuvette and the actin/$T\beta_4$ sample was added to the cuvette containing the DNaseI. This was allowed to incubate at room temperature for 10 minutes. Then 1 ml of DNA was added to the cuvette and the absorbance at 260 nm was measured every 30 seconds for 3 minutes.

Results and Conclusions

Sputum migration assays

Fig. 2A represents a typical experiment with the CF sputum. From this data it can be seen that $T\beta_4$ significantly decreased the viscosity at doses 20 ug, 40 ug, and 100 ug. The migration rate measurements at higher volumes (the 150 ug measurement) tended to skew results because of the volume of liquid added to the samples. In samples that had volume increases of over 10% of total volume, the water added decreased the viscosity of the sample. The measurement at the 60 ug sample occurred approximately every 10 samples. In this case the water control slid faster than the treated sample. This was due to the thickness of the sputum before incubation; not every sputum sample was the same density.

Despite a few inconsistencies, the $T\beta_4$ had a significant effect on the sputum samples. This preliminary data was also supported by the following data from the in vitro DNaseI assay with $T\beta_4$ and F-actin. The results can be seen in Fig. 2A.

From the Fig. 3A data it can be seen that with an increase of $T\beta_4$ there was a decreased percentage of F-actin in the sample. This was seen in a decreased activity of DNaseI. This data demonstrates the ability of $T\beta_4$ to depolymerize actin filaments. Without being bound to any particular theory, this depolymerization activity may be due to $T\beta_4$ sequestering G-actin monomers, or $T\beta_4$ may bind directly to the filament and cause its depolymerization.

According to the invention, $T\beta_4$ is believed to be effective in treating the acute and chronic lung diseases identified above, both by reducing the severity of actin toxicity in the blood (by maintaining actin in its sequestered G-actin form), and by down-regulating a number of cytokines, prostaglandin intermediates, and free radicals, which in excess are toxic and cause significant inflammation and accumulation of monocytes, neutrophils, and other cells that exacerbate tissue destruction. In preferred embodiments, $T\beta_4$ is administered by injection or by spraying $T\beta_4$ directly into the lungs.

$T\beta_4$ and $T\beta_4$ analogs, homologues and fragments having $T\beta_4$ activity appear to have the ability to both sequester actin monomers (G-actin), and down-regulate the major inflammatory cytokines such as IL-1α, IL-6, TNF-α, and PAF; as well as a number of arachidonic acid metabolites such as $Tx\beta_2$ and 6-keto-PGF1α; in addition to lipid peroxidation. The cascade of free radicals and inflammatory molecules is deleterious, and contributes to the pathology of the lung diseases described above.

$T\beta_4$, when sprayed directly into the lungs, reduces inflammation and promotes healing by down-regulating the monocytes, neutrophils, and other white blood cells that exacerbate the inflammatory process. Given intravenously or by subcutaneous or intramuscular injection, $T\beta_4$ and $T\beta_4$ analogs, homologues and fragments reduce the clogging of lung capillaries and thus prevent death and promote healing by down-regulating the inflammatory cytokines and molecules produced during this process.

Example 2

The objective was to determine the effects of $T\beta_4$ and DNase I on the properties of OAD sputum collected from six patients with stable CF lung disease.

Synthetic $T\beta_4$ was provided by Alpha 1 Biomedicals, Inc. and recombinant human DNase I (Pulmozyme®) was obtained from Genentech.

OAD sputum was analyzed untreated, and after the addition of amphibian Ringer's solution (negative control) mixed 1:5 v/v with the sputum,

Data analyses were performed using a StatView™ 4 statistics package (Abacus Concepts, Inc., Berkeley, CA) and a Power PC Macintosh® computer. The results demonstrate a synergistic effect with the combination of $T\beta_4$ and DNase I.

Without being bound to any particular theory, the synergistic effect on viscoelasticity brought about with a combination of $T\beta_4$ and DNase I may be explained by an enhanced effect of depolymerizing F-actin along with severing DNA. As DNase I also binds G-actin, which in turn inactivates DNase I activity, this synergy may also be due to enhanced DNase I activity by blocking the formation of actin-DNase I complexes.

Table 4

Means Table for G" 100
Effect: Groups

	Count	Mean	Std. Dev.	Std. Err.
Ringer's Control	6	316.898	254.448	103.878
rhDNase 30 mcg/ml	6	189.571	113.309	46.258
Tβ4 0.3 mcg/ml	6	268.569	119.712	48.872
Tβ4 3 mcg/ml	6	188.528	162.733	66.435
Tβ4 30 mcg/ml	6	176.159	141.417	57.733
Tβ4 150 mcg/ml	6	158.890	141.441	57.743
DNase + Tβ4 3+3 mcg/ml	6	147.064	135.730	55.411

Table 5

Means Table for G' 1
Effect: Groups

	Count	Mean	Std. Dev.	Std. Err.
Ringer's Control	6	332.372	272.312	111.171
rhDNase 30 mcg/ml	6	216.489	135.375	55.267
Tβ4 0.3 mcg/ml	6	268.308	189.511	77.368
Tβ4 3 mcg/ml	6	262.525	251.738	102.772
Tβ4 30 mcg/ml	6	228.752	283.664	115.805
Tβ4 150 mcg/ml	6	205.776	290.553	118.618
DNase + Tβ4 3+3 mcg/ml	6	165.150	142.101	58.013

Table 6

Means Table for G" 100
Effect: Groups

	Count	Mean	Std. Dev.	Std. Err.
Ringer's Control	6	751.615	566.221	231.159
rhDNase 30 mcg/ml	6	470.548	360.673	147.244
Tβ4 0.3 mcg/ml	6	633.453	362.845	148.131
Tβ4 3 mcg/ml	6	464.464	427.055	174.344
Tβ4 30 mcg/ml	6	430.879	429.886	175.500
Tβ4 150 mcg/ml	6	438.765	550.637	224.796
DNase + Tβ4 3+3 mcg/ml	6	286.722	330.983	135.123

While the invention has been described and illustrated with details and references to certain preferred embodiments, those skilled in the art will appreciate that various modifications, changes, omissions, and substitutes can be made without departing from the spirit of the invention.

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 1:

Ser Asp Lys Pro Asp Met Ala Glu Ile Glu Lys Phe Asp Lys Ser Lys
1 5 10 15

Leu Lys Lys Thr Glu Thr Gln Glu Lys Asn Pro Leu Pro Ser Lys Glu
20 25 30

Thr Ile Glu Gln Glu Lys Gln Ala Gly Glu Ser
35 40

(3) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 2:

Ser Asp Lys Pro
1

(4) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 3:

Lys Leu Lys Lys Thr Gly Thr
1 5

(5) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 8:

```
Ala Asp Lys Pro Asp Met Ala Glu Ile Glu Lys Phe Asp Lys Ser Lys
1               5                  10                  15

Leu Lys Lys Thr Glu Thr Gln Glu Lys Asn Pro Leu Pro Ser Lys Glu
            20                  25                  30

Thr Ile Glu Gln Glu Lys Gln Ala Gly Glu Ser
        35                  40
```

(10) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 9:

```
Ser Asp Lys Pro Asp Met Ala Glu Ile Glu Lys Phe Asp Lys Ser Lys
1               5                  10                  15

Leu Lys Lys Thr Glu Thr Gln Glu Lys Asn Pro Leu Pro Ser Lys Glu
            20                  25                  30

Thr Ile Glu Gln Glu Lys Gln Ala Gly Glu Ser
        35                  40
```

(11) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 10:

(14) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 13:

Ser Asp Lys Pro Asn Leu Glu Glu Val Ala Ser Phe Asp Lys Thr Lys
1 5 10 15

Leu Lys Lys Thr Glu Thr Gln Glu Lys Asn Pro Leu Pro Thr Lys Glu
20 25 30

Thr Ile Glu Gln Glu Lys Gln Ala Ser
35 40

(15) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 14:

Ser Asp Lys Pro Asp Leu Ala Glu Val Ser Asn Phe Asp Lys Thr Lys
1 5 10 15

Leu Lys Lys Thr Glu Thr Gln Glu Lys Asn Pro Leu Pro Thr Lys Glu
20 25 30

Thr Ile Glu Gln Glu Lys Gln Ala Thr Ala
35 40

(16) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41
(B) TYPE: Amino Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO. 15:

What is claimed is:

1. A method of reducing viscoelasticity of sputum of obstructive airway disease (OAD), comprising contacting OAD sputum with a viscoelasticity-reducing amount of Thymosin β_4 ($T\beta_4$) or a combination of $T\beta_4$ and DNase I.

2. The method of claim 1, wherein said $T\beta_4$ or said combination of $T\beta_4$ and DNase I are present in a pharmaceutical formulation including a pharmaceutically-acceptable liquid carrier.

3. The method of claim 2, further including the step of administering the pharmaceutical formulation to an OAD patient by introducing said formulation into an airway of said patient, so as to contact said formulation with said sputum.

4. The method of claim 3, wherein said pharmaceutical formulation is in aerosol form.

5. The method of claim 4, wherein said $T\beta_4$ and said DNase I are present in said pharmaceutical formulation in a respective ratio of from about 1:2 to about 2:1.

6. The method of claim 4 or 5, wherein the concentration of said $T\beta_4$ in said formulation is within the range of from about 0.1 mcg/ml to about 10 mg/ml, or the concentrations of said $T\beta_4$ and said DNase I in said formulation are each within the range of from about 0.1 mcg/ml to about 10 mg/ml.

7. The method of claim 6, wherein said range is about 0.1-10 mg/ml.

17. The pharmaceutical formulation of claim 16, wherein said range is about 0.3-7 mg/ml.

18. The pharmaceutical formulation of claim 17, wherein said range is about 0.5-5 mg/ml.

19. The pharmaceutical formulation of claim 16, wherein said range is about 0.1-10 mg/ml, said ratio is about 1:1 and said pharmaceutical formulation is in aerosol form.

20. The pharmaceutical formulation of claim 19, wherein said range is about 0.5-5 mg/ml.

Thymosin β_4*Ala

Ac-Ala-Asp-Lys-Pro-Asp-Met-Ala-Glu-Ile-Glu-Lys-Phe-Asp-Lys-Ser-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-Glu-Lys-Asn-Pro-Leu-Pro-Ser-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Gln-Ala-Gly-Glu-Ser-OH

Thymosin β_4-Xen

Ac-Ser-Asp-Lys-Pro-Asp-Met-Ala-Glu-Ile-Glu-Lys-Phe-Asp-Lys-Ala-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-Glu-Lys-Asn-Pro-Leu-Pro-Ser-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Gln-Thr-Ser-Glu-Ser-OH

Thymosin β_9

Ac-Ala-Asp-Lys-Pro-Asp-Leu-Gly-Glu-Ile-Asn-Ser-Phe-Asp-Lys-Ala-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-Glu-Lys-Asn-Thr-Leu-Pro-Thr-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Gln-Ala-Lys-OH

Thymosin β_9-MET

Ac-Ala-Asp-Lys-Pro-Asp-Met-Gly-Glu-Ile-Asn-Ser-Phe-Asp-Lys-Ala-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-Glu-Lys-Asn-Thr-Leu-Pro-Thr-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Gln-Ala-Lys-OH

Thymosin β_{10}

Ac-Ala-Asp-Lys-Pro-Asp-Met-Gly-Glu-Ile-Ala-Ser-Phe-Asp-Lys-Ala-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-Glu-Lys-Asn-Thr-Leu-Pro-Thr-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Arg-Ser-Glu-Ile-Ser-OH

FIG. 1B

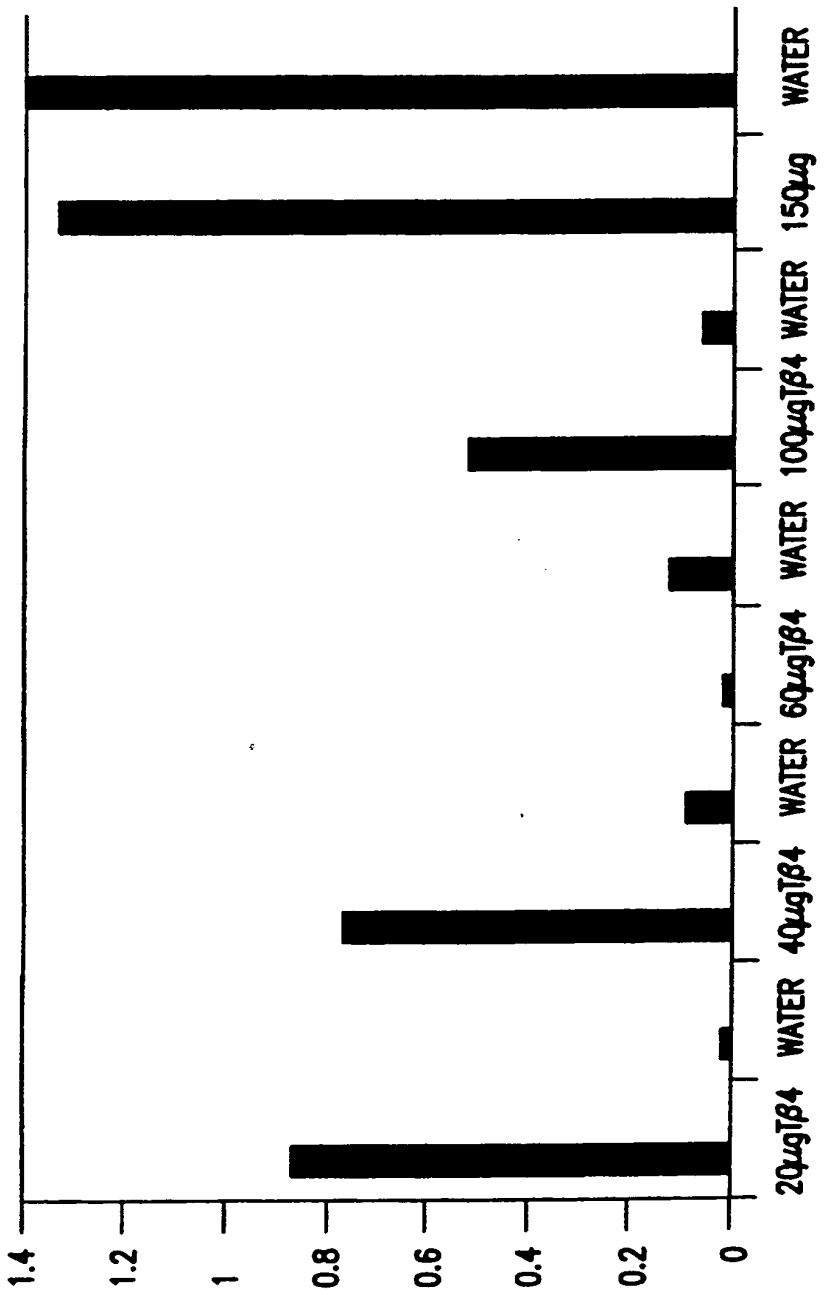

1.4
1.2
1
0.8
0.6
0.4
0.2
0
20μgTβ4
WATER
40μgTβ4
WATER
60μgTβ4
WATER
100μgTβ4
WATER
150μg
WATER

FIG.2A

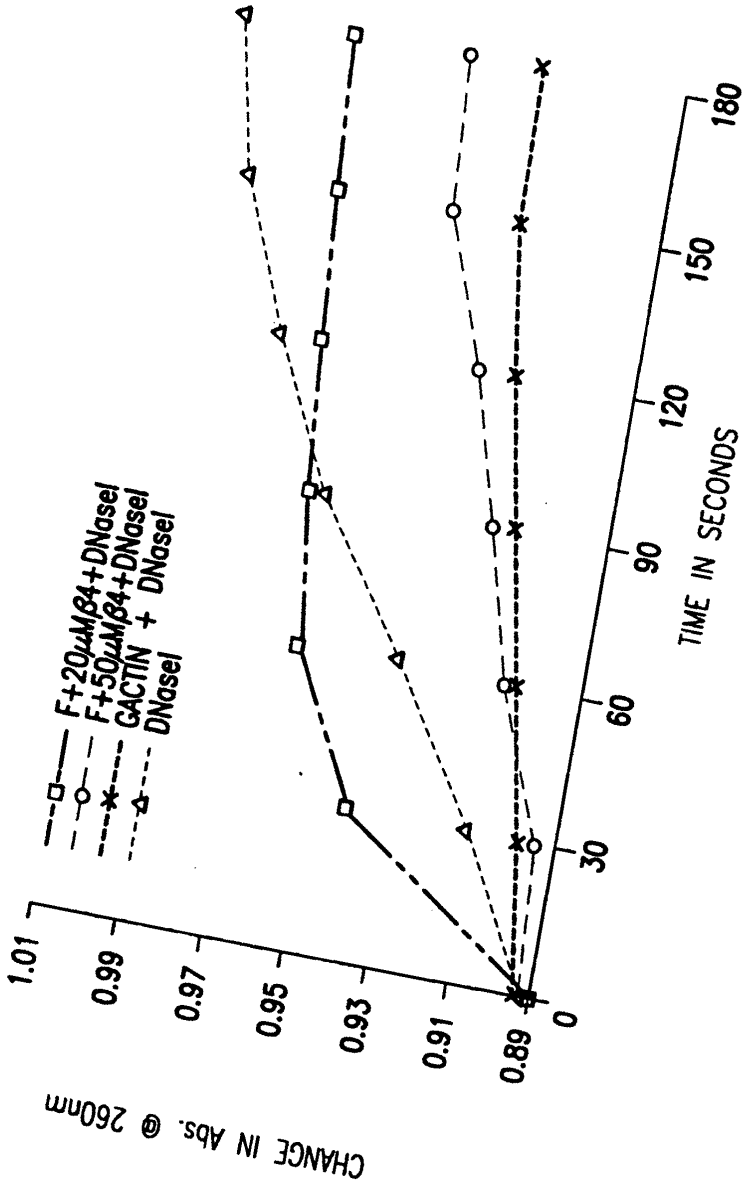

F+20μMβ4+DNaseI
F+50μMβ4+DNaseI
GACTIN + DNaseI
DNaseI
CHANGE IN Abs. @ 260nm
1.01
0.99
0.97
0.95
0.93
0.91
0.89
0
30
60
90
120
150
180
TIME IN SECONDS

FIG. 3A

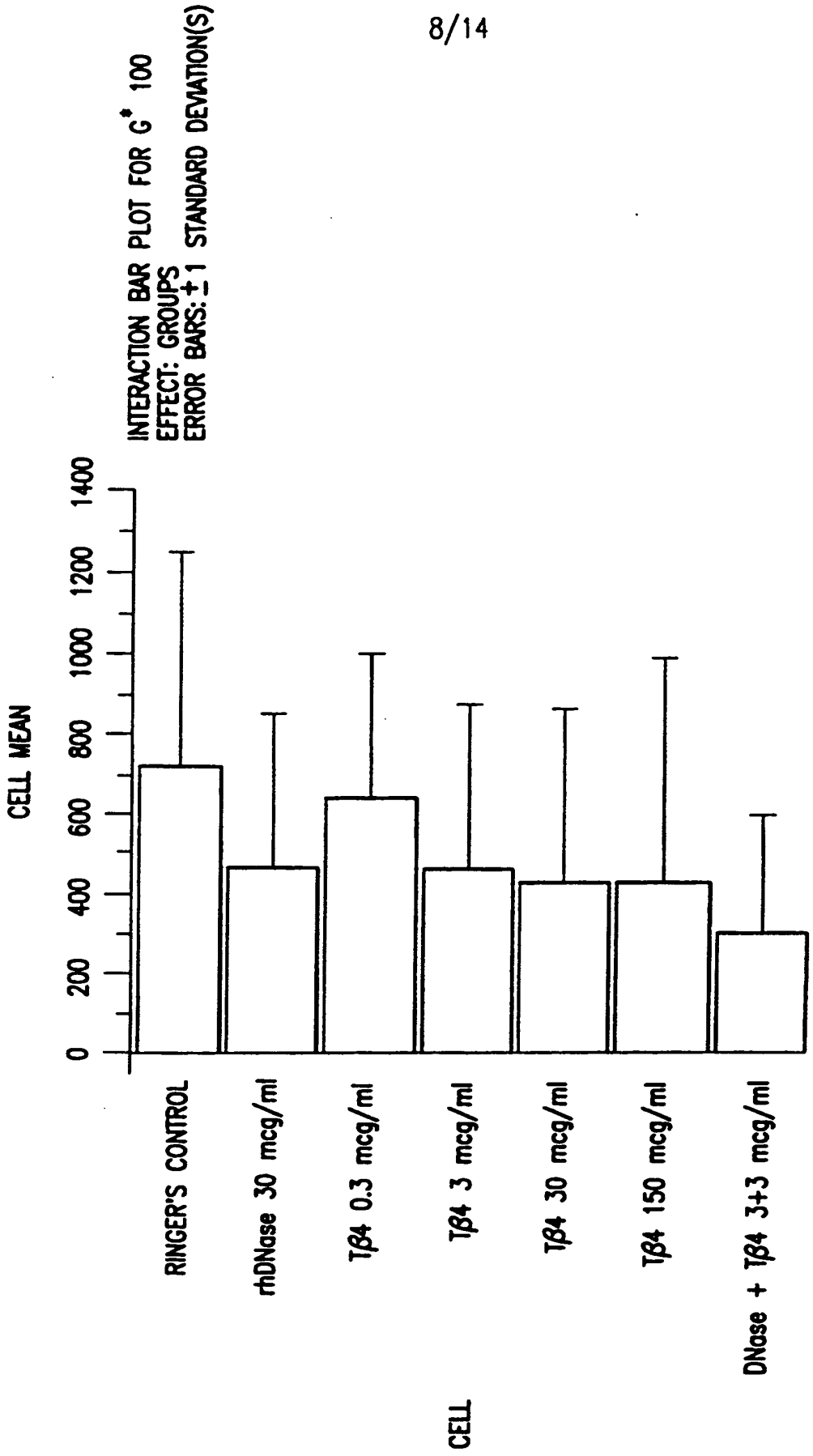
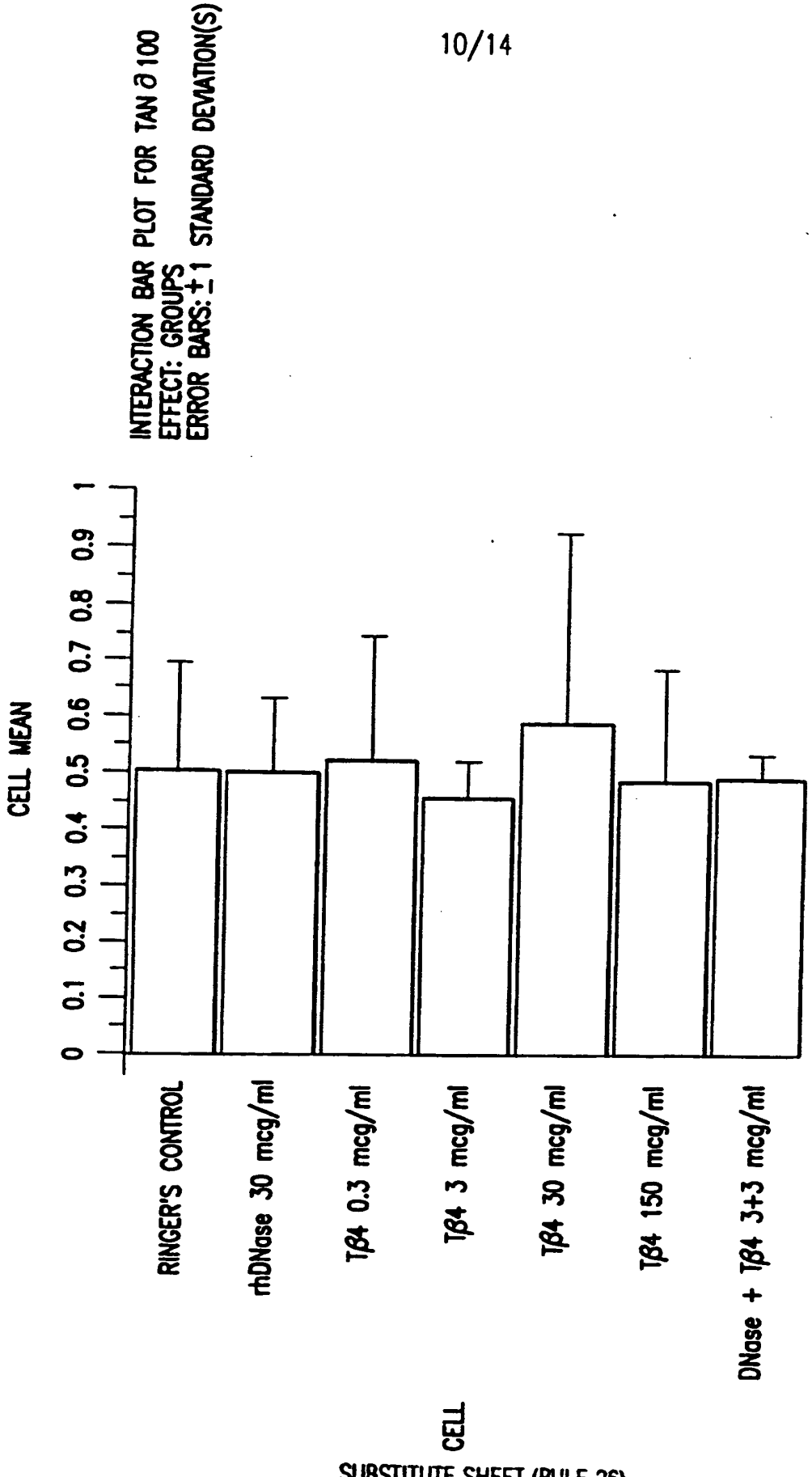

INTERACTION BAR PLOT FOR G* 100
EFFECT: GROUPS
ERROR BARS: +-1 STANDARD DEVIATION(S)
CELL MEAN
0
200
400
600
800
1000
1200
1400
RINGER'S CONTROL
rhDNase 30 mcg/ml
Tβ4 0.3 mcg/ml
Tβ4 3 mcg/ml
Tβ4 30 mcg/ml
Tβ4 150 mcg/ml
DNase + Tβ4 3+3 mcg/ml
CELL

FIG.4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EFFECT: GROUPS
ERROR BARS: +1 STANDARD DEVIATION(S)
CELL MEAN
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0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
RINGER'S CONTROL
rhDNase 30 mcg/ml
Tβ4 0.3 mcg/ml
Tβ4 3 mcg/ml
Tβ4 30 mcg/ml
Tβ4 150 mcg/ml
DNase + Tβ4 3+3 mcg/ml
CELL

FIG.6

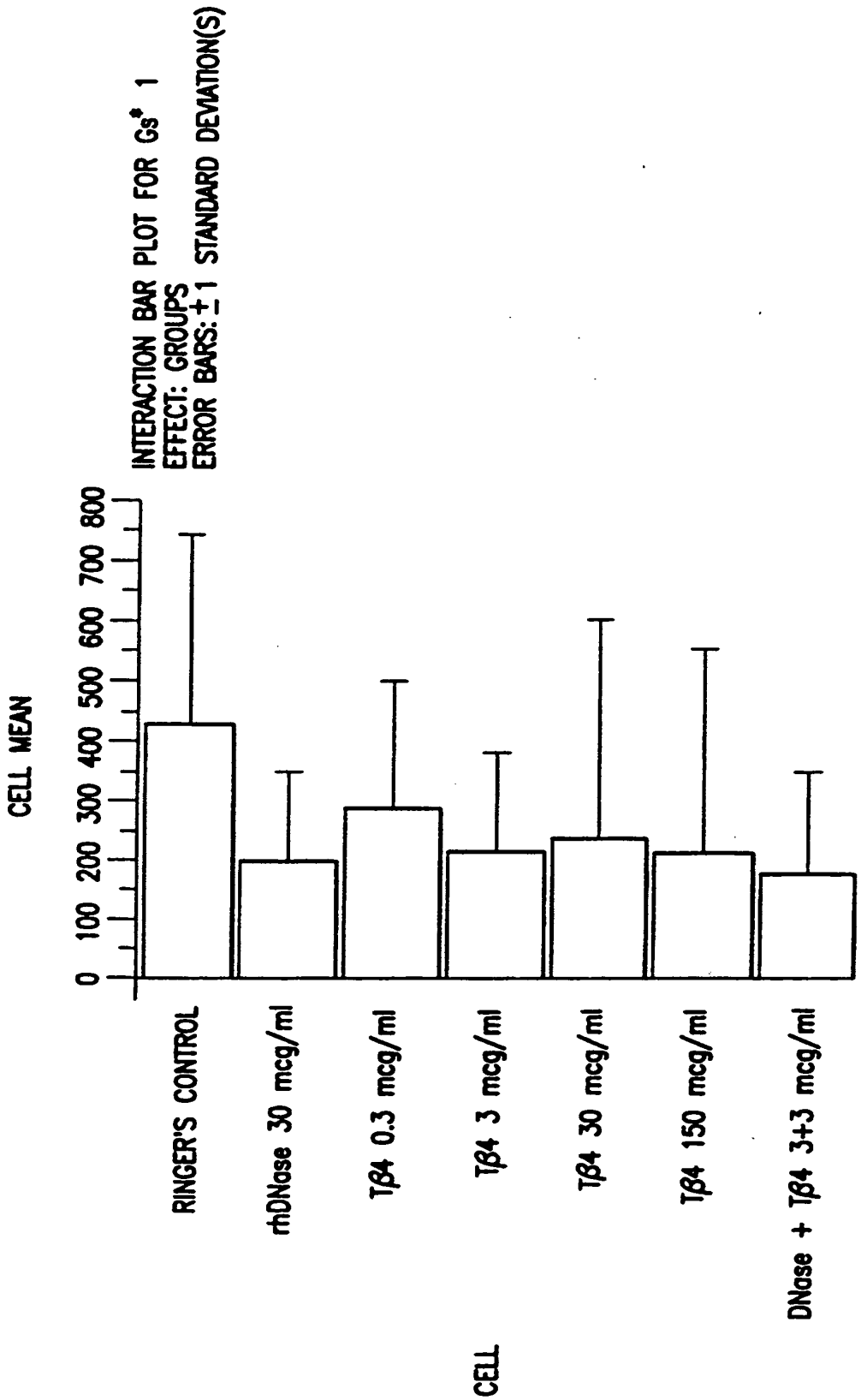
INTERACTION BAR PLOT FOR Gs* 1
EFFECT: GROUPS
ERROR BARS: +-1 STANDARD DEVIATION(S)
CELL MEAN
0 100 200 300 400 500 600 700 800
RINGER'S CONTROL
rhDNase 30 mcg/ml
Tβ4 0.3 mcg/ml
Tβ4 3 mcg/ml
Tβ4 30 mcg/ml
Tβ4 150 mcg/ml
DNase + Tβ4 3+3 mcg/ml
CELL

FIG.8

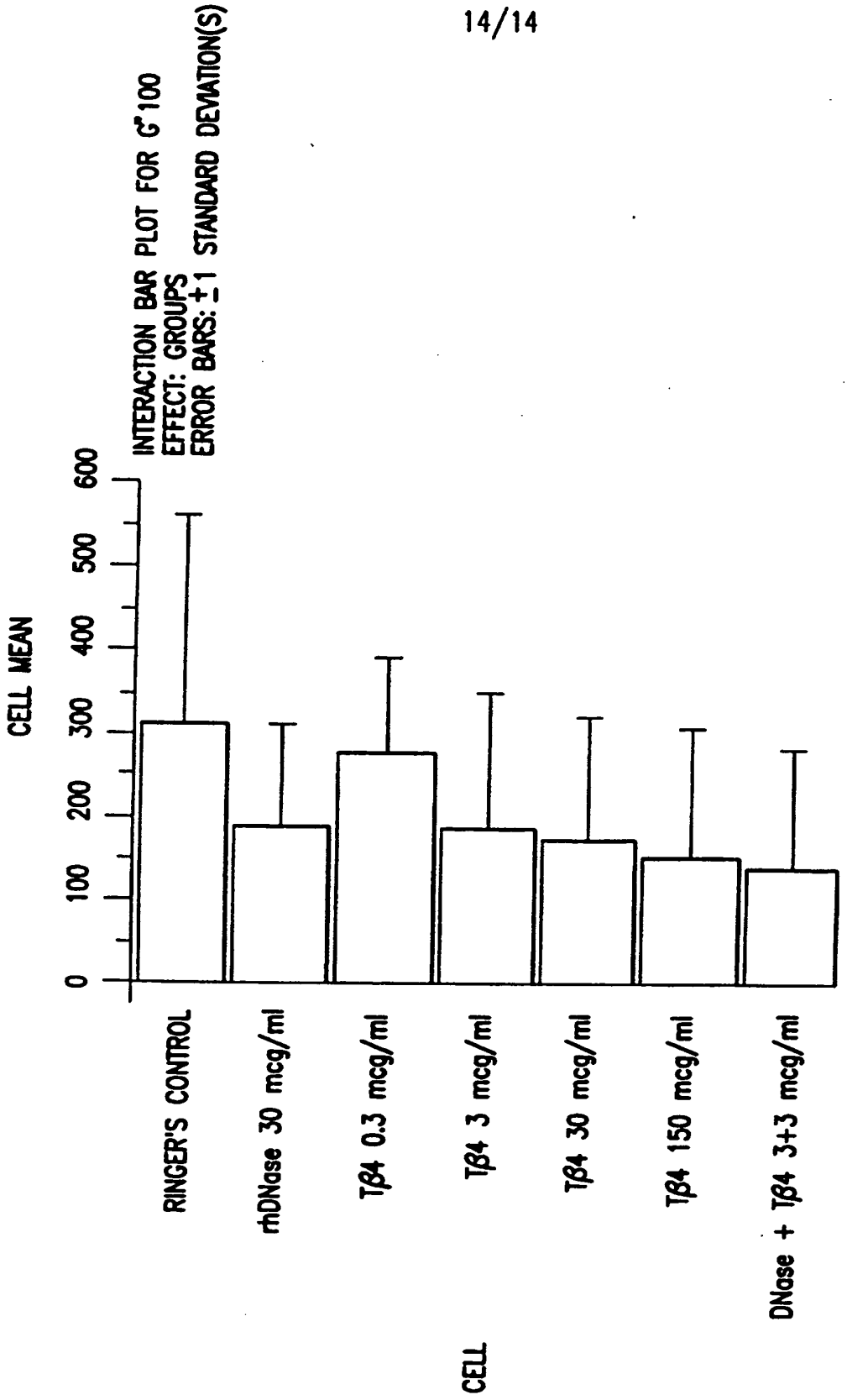
INTERACTION BAR PLOT FOR G*100
EFFECT: GROUPS
ERROR BARS: +1 STANDARD DEVIATION(S)
CELL MEAN
0
100
200
300
400
500
600
RINGER'S CONTROL
rhDNase 30 mcg/ml
Tβ4 0.3 mcg/ml
Tβ4 3 mcg/ml
Tβ4 30 mcg/ml
Tβ4 150 mcg/ml
DNase + Tβ4 3+3 mcg/ml
CELL

FIG.10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/12543

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation f document, with indication, where appropriate, of the relevant passages	Relevant to claim N .
Y	New England Journal of Medicine, Volume 326, Number 26, Lee et al, "The extracellular actin-scavenger system and actin toxicity", pages 1335-1341, see entire article.	1-20